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Determination of Low Levels of Bupivacaine (Marcaine®) in Plasma During Epidural Analgesia

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Summary: A rapid method is described for the determination of plasma levels of bupivacaine used for epidural analgesia during childbirth. Data obtained in a clinical study are also given.

Bestimmung von niedrigen Bupivacain (Marcain®)-Konzentrationen im Plasma während Epiduralanalgesie

Zusammenfassung: Es wird eine schnelle Methode zur Bestimmung des Bupivacains im mütterlichen Blut bei epiduraler Analgesie während des Geburtvorgangs beschrieben. Die ermittelten klinischen Daten werden ebenfalls aufgeführt.

Introduction

The increasing demand for epidural analgesia during childbirth focusses attention on the possible side effects of the analgesic drug in mother and child. Estimation of drug levels in maternal and foetal (umbilical cord) plasma serves to supervise the degree of leakage from the application site. The drugs used in this type of analgesia are potent myocardial suppressants and they are able to cause a constriction of the uterine vessels, in this way decreasing the utero-placental bloodflow (1). A correlation can also be made between cardiac activity of the child and uterine contraction in the mother (cardiotocography) as well as with the general condition of the newborn, e. g. Apgar score, etc. (2).

In the obstetric department of our hospital a prospective study was started on the influence of lumbar epidural analgesia with bupivacaine-adrenaline on the maternal and foetal heart rate patterns.

Methods described hitherto for the determination of bupivacaine in plasma either require large blood samples and special equipment (3, 4), or lack sensitivity (5), and without exception are rather laborious. A high sensitivity, however, is required because modern techniques of anaesthesia permit the use of relatively low doses of the drug with correspondingly low plasma levels. In this communication we present a modified and rapid estimation procedure using active charcoal for the adsorption of bupivacaine from acidified plasma.

Materials and Methods

Reagents

1. Active charcoal (BDH Chemicals Ltd, cat. number 33032). A suspension of 0.4 g in 0.1 litre 0.75 mol/l HCl is prepared before use.

2. Dichloromethane (analytical grade).

3. Standard solution of bupivacaine · HCl, 50 mg/l.

Extraction

In a 10 ml centrifuge tube 1 ml of serum is mixed with 2 ml of charcoal suspension, vortexed for 30 seconds and centrifuged 2 minutes. The supernatant is removed by suction, 1 ml of dichloromethane is added, mixed for 45 seconds (Vortex) and filtered over glass wool into a 3 ml tube. The charcoal is rinsed with 1 ml of dichloromethane (Vortex) and the combined organic extracts are evaporated to dryness in a nitrogen stream at 40 °C.

The residue in the tube is dissolved in 20 µl of water and 2 µl of this solution are applied on the gas chromatography column.

Gas chromatography

A Packard-Becker chromatograph model type 427 is used with normal flame ionisation detector, equipped with a silanized spiral glass column, length 180 cm, internal diameter 0.2 cm, packed with 3% SE 30 on Gaschrom Q (100/120 mesh) (Applied Science lab.).

Temperatures: oven 250 °C, detector 300 °C, injection channel 300 °C.

Carrier gas: nitrogen 2.7 kg/cm^2 (40 p.s.i.).

Attenuation 10 × 8.

The standard solution (50 mg/l bupivacaine · HCl) is diluted tenfold with water prior to use. A sample of 2 µl of the diluted

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standard (thus containing 10 ng bupivacaine · HCl) is applied directly on the column.

Calculation

$$\frac{\text{peak height sample}}{\text{peak height standard}} \times 8.9 \times \frac{100}{64} \times 10 = \mu g/l \text{ bupivacaine}$$

Standard 10 ng bupivacaine hydrochloride in 2 µl corresponds to 8.9 ng of the free base; recovery in extraction is 64% (tab. 1); dilution factor of sample is 10.

Clinical study

As site of puncture for the administration of the drug the third-lumbar interspace was used and a catheter was introduced 5 cm upwards. We injected 10 ml bupivacaine 2.5 g/l with adrenaline 1/200,000 through the catheter. Every two hours a topping-up dose of 8 ml of this anaesthetic solution was given. The study was performed during spontaneous labour in healthy nulliparous full-term patients with uncomplicated pregnancies.

For the determination of bupivacaine, blood samples were collected from the ante-cubital vein viz. 10, 15, 30, 45, 90 and 120 minutes after the first epidural injection. Foetal E.C.G., maternal E.C.G. and intra-uterine pressure were recorded on a strip-chart-recorder and magnetic tape and processed offline in a Digital computer system. In one group of 13 patients receiving epidural analgesia several calculated parameters could be compared before and following the administration of the analgesic. Another group of 12 patients was studied following the same protocol without epidural analgesia. All patients were in the lateral position.

Tab. 1. Charcoal extraction of added bupivacaine · HCl from pooled human plasma. Samples were analysed in duplicate on six consecutive days.

Bupivacaine · HCl added (µg/l)	n	% Recovery (%)	C. V. (%)
50	12	57	5.0
100	12	64	4.3
200	12	66	3.8
300	12	69	3.6
mean recovery		64	4.2

Results

Technical

The procedure described is a modification and combination of techniques found elsewhere and it results in an easy determination of bupivacaine in human blood serum or plasma. The efficacy of the method is especially due to the extraction procedure. Bupivacaine is extracted from the acidified plasma by charcoal adsorption (tab. 1) with an efficiency of 64% in the range between 50–300 µg/l. In such procedures the co-adsorption of organic material is often a cause of much interference in the final detection. To overcome this problem the organic matter from charcoal is dissolved in dichloromethane and evaporated to dryness. From this residue, bupivacaine easily extracted with water, leaving behind much organic material which could cause interference. In this way a "clean" chromatogram is easily obtained (fig. 1).

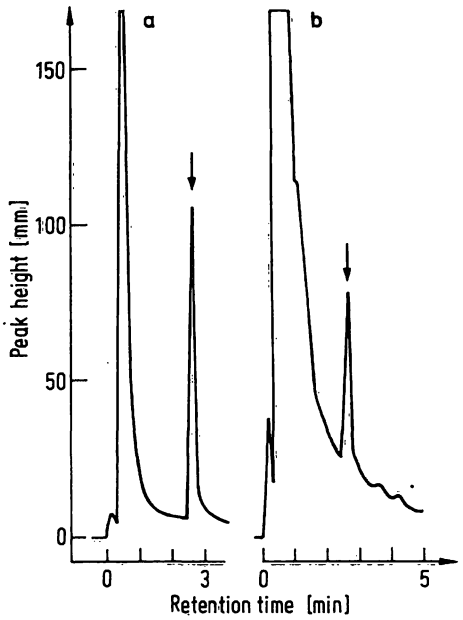

Fig. 1. Chromatograms of bupivacaine hydrochloric standard solution, 10 ng applied on column (a), and serum extract (b).

There is a linear relation between peak height and concentration.

The use of an internal standard is not necessary provided the analysis is performed in duplicate (C. V. 5% from a series of duplicates n = 80).

In incidental cases where only one estimation for the patient is required an external standard of bupivacaine hydrochloride in plasma is prepared. In serial studies, however, this would require too much blood from the patient. In any analysis or series thereof a patient's blank is prepared from blood taken before the first estimation of the drug, thus avoiding false results from concomitant medication. The detection limit (twice the patient's blank value) is 5 µg/l.

Clinical

After epidural analgesia a minor decrease in the foetal heart rate of about 2–4 beats per minute was found. In the last $1\frac{1}{2}$ hours of the first stage, we observed a decrease in the beat-to-beat variations of the foetal heart rate in the epidural group. It is our opinion that there might be a correlation between these changes in foetal heart rate patterns and the bupivacaine levels.

The bupivacaine levels in 13 patients were measured and the results given in figure 2.

Discussion

Because the time required for a single analysis is only about 15 minutes, the method is most suitable for monitoring blood levels, even during labour.

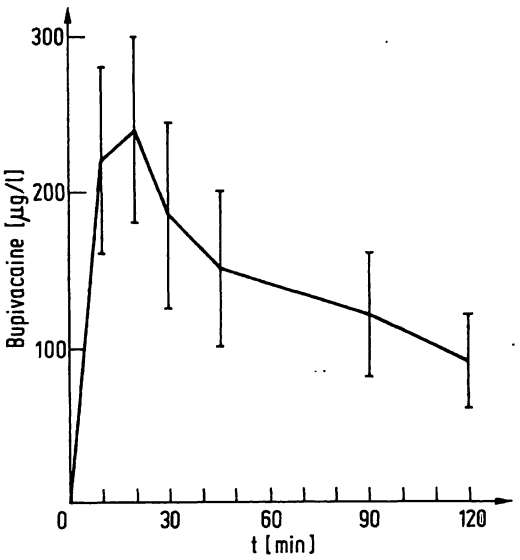

Fig. 2. Bupivacaine levels (means ± s.d.) in maternal blood plasma after administration of 10 ml 2.5 g/l solution (25 mg) of the drug; the solution contains also adrenalin 5 mg/l.

The rather small amount of plasma needed for the estimation and the rapid availability of results is certainly of great help to the obstetrical department. The administration of bupivacaine can be correlated with the plasma levels in the maternal blood. Overloading the patients can be prevented in this way. Another advantage is that changes in the cardiotocogram can be considered in combination with bupivacaine levels, thus distinghuishing the administration of bupivacaine from other causes of change in foetal heart rate patterns.

At present a comparison is being made between larger groups with and without bupivacaine during labour. Results of this clinical study with more detailed information about the obstetrical data will be reported elsewhere.

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